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The density dependence of plant responses to elevated CO_2

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Summary

1 Stands of the annual *Brassica kaber* were grown at a range of six densities in both ambient and elevated CO_2 environments, and measurements of shoot growth were made from seedling emergence through to reproduction.
2 Early in stand development (21 days following emergence), CO_2 enhancement (β) for above-ground biomass was highly density-dependent, ranging from 1.41 at the lowest density (20 plants m^{-2}) to 0.59 at the highest density (652 plants m^{-2}).
3 As stands matured and total biomass exceeded a relatively low threshold level (< 10.0 g m^{-2}; *c.* 20% of final yield), the density-dependence of β disappeared. Above this shoot biomass threshold, β-values remained remarkably stable ($\beta = 0.34$) across a broad range of stand biomass, independent of a stand's initial density or age.
4 Average stand-level reproductive β-values at a final harvest were very similar to biomass values ($\beta = 0.38$) and, as with biomass values at later stages, showed no apparent density-dependence.
5 These results highlight the importance of considering density and the time-course of stand development simultaneously when assessing the potential for CO_2-induced growth enhancements in plants.

Keywords: Brassica kaber, CO_2 responsiveness, competition, stand development

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Introduction

The current anthropogenically induced rise in the concentration of atmospheric CO_2 has the potential to increase plant productivity significantly (Strain & Cure 1985; Bazzaz *et al.* 1995a; Ciais *et al.* 1995; Houghton *et al.* 1996). Recent literature reviews suggest that an increase in CO_2 concentration to double the current levels would result in average biomass enhancements of 30–40% (Kimball 1983; Cure & Acock 1986; Hunt *et al.* 1991; Lawlor & Mitchell 1991; Poorter 1993; Ceulmans & Mousseau 1994; Wullschleger *et al.* 1995). However, these estimates are based largely on studies characterizing the responses of individually grown plants that have been raised in the absence of competition. Improved predictions of the effects of elevated CO_2, and the consequences of the potential increases in productivity for terrestrial communities and ecosystems, requires that the influences of neighbouring plants are considered (Woodward *et al.* 1991; Bazzaz & McConnaughay 1992; Korner & Bazzaz 1996).

Studies that have incorporated such density-dependent interactions suggest that CO_2-induced growth enhancements are generally lower when individuals are grown in the presence of neighbouring plants (du Cloux *et al.* 1987; Ackerly & Bazzaz 1995; Bazzaz *et al.* 1995b; Retuerto *et al.* 1996). For instance, yellow birch seedlings exposed to elevated CO_2 concentrations increased in biomass by 49% when grown individually but only by 14% when grown in dense stands (Wayne & Bazzaz 1995, 1997). However, such studies offer only limited insight into the effects of density-dependent processes on CO_2 responsiveness for two reasons. First, the designs of these experiments rarely include more than two densities, one of which is individually grown plants, and secondly, studies investigating the interactions between density and CO_2 rarely characterize growth responses over the time–course of individual-plant or whole-stand ontogeny.

The quantitative relationship between plant density and productivity has been well characterized by population biologists (Harper 1977; Silvertown &

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Lovett-Doust 1993). During the early stages of stand development, before severe interference between plants occurs, higher initial density generally results in progressively higher yield (e.g. biomass). However, as neighbouring plants grow, they occupy space and consume resources that become limiting and subject to competition. During this stage of stand development, therefore, incremental increases in initial density lead to progressively smaller gains in yield and, beyond a certain threshold density, no additional increase in yield is found. This asymptotic relationship between plant density and yield is commonly referred to as the law of constant final yield (Kira *et al.* 1953; Shinozaki & Kira 1956; De Wit 1960; Watkinson 1980). Because of this non-linear density–yield relationship, it is difficult to extrapolate the responses of plant populations to CO_2 at other plant densities from studies employing single plants and only one higher density.

The importance of making sequential observations during stand development for understanding the processes of interference has long been acknowledged (Milthorpe 1961; Connolly *et al.* 1990; Weiner 1990), and is especially relevant for understanding the density-dependent nature of CO_2 responsiveness. Growth analysis studies of individual plants suggest that, in most cases, the magnitude of the direct effects of CO_2 varies considerably through time (Bazzaz 1993; Loehle 1995). Many species show decreasing photosynthetic and growth responsiveness to CO_2 over time, a phenomenon broadly referred to as acclimation (DeLucia *et al.* 1985; Arp 1991; Bowes 1991; Bazzaz *et al.* 1993). Because both density-dependent interactions and the magnitude of growth stimulation caused by elevated CO_2 vary through time, sequential observations are necessary to characterize accurately the responses of plant populations to CO_2.

We made three predictions regarding the interaction between plant density and CO_2 on stand development. First, the magnitude of CO_2-induced growth enhancement during early stages of stand development will decrease with increasing densities, as resource limitation is likely to increase with density. Secondly, the effects of density on the magnitude of CO_2-induced growth enhancement will decrease with time, as standing biomass across all density treatments at a CO_2 concentration converge towards a higher yield. Finally, as increases in both stand age and plant density result in greater stand-level biomass, and because stand-level biomass is what largely determines rates of resource depletion, we expect that increases in total stand biomass will result in a decreased response to CO_2, independent of a stand's age or density.

To test these predictions, we conducted a controlled environment study to investigate the response of the annual plant *Brassica kaber* to a range of six densities, in both ambient ($350\,\mu l\ l^{-1}$) and elevated ($700\,\mu l\ l^{-1}$) CO_2 atmospheres. Both shoot biomass and total leaf area were measured at three sequential harvests. Reproductive yield and root mass were estimated at the final harvest. In this paper we characterize the combined effects of density, CO_2, and time on stand-level productivity. A later companion paper will focus on the effects of these factors on individual plant variability and stand size structure.

Materials and methods

SPECIES, GROWTH CONDITIONS AND MEASUREMENTS

Brassica kaber var. *pinnafitida* (Stokes) L. C. Wheeler (field mustard) was chosen for study because of its importance as an agricultural weed in the mid-western regions of the USA (Slife *et al.* 1960). Seed of *B. kaber* was collected from a population in Woodstock, Illinois (F & J Seed Service, Woodstock, IL). On 23 December 1996, seed was sown directly into 5.5-l round pots (25 cm diameter) filled with a 2 : 1 mixture of Promix BX (Premier Horticultural Inc., Redhill, PA) and horticultural washed sand. Seed was sown at six densities: 1, 2, 4, 8, 16 and 32 plants pot^{-1}, corresponding to field densities of *c.* 20, 41, 81, 163, 326 and 652 plants m^{-2}. Seed within all pots were equally spaced and at the two highest densities were sown in a regular hexagonal design. To eliminate the need for transplanting, and to minimize initial variation in seedling emergence time (and thus seedling size), numerous seeds were sown at each desired seedling location. Five days after cotyledons began to emerge, seedlings were thinned to one seedling per location, choosing seedlings of similar size within and between pots within each CO_2 treatment.

Growing temperatures were maintained at $26 \pm 2.0\,^{\circ}C$ during the day and $21 \pm 2.0\,^{\circ}C$ at night. Plants were watered regularly, as needed, and received glasshouse light (*c.* 70% of full sun) supplemented with light from metal halide lamps. Plants were fertilized on 10 January and 20 January 1997 with 20 ml of full strength Peter's Solution (Scott-Sierra Horticultural Products Co., Marysville, OH). To minimize edge effects that might have affected all but the lowest density pots, a collar of neutral density shade cloth was placed around all pots, held up with wooden stakes. The height of the shade cloth collar was regularly adjusted to match average canopy height within a pot. Within modules, pot locations were randomized approximately every 10 days.

The experiment was laid out as a split-plot design with six main plots structured as three blocks, each with two CO_2 concentrations (350 and $700\,\mu l\ l^{-1}$). Each main plot contained 42 stands (subplots), with all combinations of six densities × three harvest times replicated twice (except those at the lowest density, which were replicated four times).

Harvest 1 (H1) was on 18 January 1997, when canopies of plants at high density had just begun to

overlap physically [leaf area index (LAI) = 0.4 and 0.2 for ambient and elevated CO_2 treatments, respectively]. Harvest 2 (H2) was on 27 January, when plant canopies at high density were well developed (LAI = 2.6 and 2.9) but only a few individuals had initiated flowering. A final harvest (H3) was on 17 February, when a large number of flowers had matured into fruits but before many leaves had senesced (LAI = 2.9 and 2.1). In addition to these three harvests, an initial harvest (H0) was conducted on 3 January, 12 days after germination, using a total of 18 additional seedlings per CO_2 treatment grown individually in 0.2-l pots filled with the same soil described above. This harvest was used to assess the initial effects of CO_2 on seedling growth prior to any density-dependent interactions and to estimate relative growth rate prior to H1.

At each harvest, leaf areas were measured for all plants. At H1–H3 a Licor leaf area meter (Licor, Nebraska, USA) was used. For the small plants of H0, cotyledons and leaves were laid out flat between two sheets of acetate, photocopied, and areas were then estimated from these images using a flat bed scanner and an image processing program. After partitioning into leaves and support structures (stem and petioles), shoot material was oven dried at 70 °C for 1 week, and then weighed. At H3, additional below-ground measures were made to assess the degree to which shoot mass responses to CO_2 and density reflect qualitatively and quantitatively the responses of whole plant mass. Roots were washed free of soil, and weighed as above. Finally, at H3, the total number and dry weight of all reproductive structures (flowers and fruits) in each stand were also measured.

STATISTICAL ANALYSIS AND MODELLING

An initial ANOVA of stand shoot mass and total leaf area indicated heteroscedasticity, with variance increasing with the mean of total stand response. Transforming to the log scale reduced but did not completely eliminate heteroscedasticity, there being some effects of density and harvest on residual variance (analysis not shown). There was little evidence that the main plot residual variation exceeded that at the subplot level, so the split-plot nature of the design was ignored in subsequent analyses. The logarithm of total shoot biomass (LTB) and the logarithm of total leaf area (LTLA) were analysed using a weighted analysis to allow for the heteroscedasticity, with weights predicted from an analysis of the variability of residual variation over density and harvest.

Above-ground relative growth rates (RGR) were calculated for each interharvest period in all treatments. Above-ground plot mass at H0 was estimated by multiplying values for individually grown seedlings raised in either ambient or elevated CO_2 environments by the appropriate plot density. The model described above was used to predict mean log shoot biomass for each density × harvest × CO_2 level and the standard errors of these values. The means were then used to calculate RGR:

$$\mathrm{RGR} = (\log_e W_2 - \log_e W_1)/(t_2 - t_1)$$

The standard error of differences (SED) between predicted RGR values for ambient vs. elevated CO_2 was calculated from the SE for these values.

Modelling CO_2 enhancement

Results of the preliminary analysis of LTB and LTLA and additional exploratory scatterplots (plots not shown but trends similar to those in Fig. 2) suggested that for both LTLA and LTB, a relatively simple relationship independent of initial density or harvest existed between growth at ambient CO_2 and the magnitude of the CO_2 enhancement. For LTLA, the CO_2 effect appeared to decrease in a linear manner with increasing leaf area, approaching zero after a threshold leaf area was attained. For LTB, the pattern was somewhat more complex, suggesting an initial decrease in the magnitude of CO_2 enhancement with increasing shoot biomass, but eventually stabilizing to a fixed level of enhancement above a threshold biomass. These preliminary results suggested a more formal modelling approach for characterizing the magnitude of CO_2 enhancement based on expected stand-level leaf area or shoot biomass at ambient CO_2. The motivation for, and strength of, these models is in their simplicity, reducing an initially complex statistical model to a set of fewer parameters that are easier to interpret. The models are constructed on the log scale.

The response of LTLA at ambient CO_2 (L_{Aij}) to block, density and harvest can be characterized by the standard model for a two-way ANOVA in a randomized block design:

$$E(L_{Aij}) = \mu + t_i + b_j \quad (1)$$

where $E(L_{Aij})$ is the expected (population) response of LTLA at ambient CO_2; b_j denotes the jth block, and t_i is the ith harvest × density combination (there are 18 such combinations).

We assumed that LTLA under elevated CO_2 (L_{Eij}) was greater than under ambient CO_2 by an amount that declined linearly as the ambient response $E(L_{Aij})$ increased. This can be written in terms of the usual beta factor (β), which expresses the magnitude of the CO_2 response:

$$\log(1+\beta) = E(L_{Eij}) - E(L_{Aij}) = \alpha + \gamma\, E(L_{Aij}) = \alpha + \gamma(\mu + t_i + b_j) \quad (2)$$

after substituting in equation 1.

For LTB the CO_2 effect appears to be biphasic, and the model proposed above was modified to include a threshold above which β was constant. Biomass responses at ambient (B_{Aij}) and elevated (B_{Eij}) CO_2 are

defined by analogy with those for leaf area. $E(B_{Aij})$ can be expressed as a simple randomized block model similar to equation 1.

The first phase of the model, at low biomass under ambient CO_2, is similar to the linear model for LTLA:

$$\log(1+\beta) = E(B_{Eij}) - E(B_{Aij}) = \alpha + \gamma E(B_{Aij}) \quad (3)$$

where $E(B_{Aij})$ can be replaced by the simple randomized block model as in equation 1. In the second phase, when the expected response at ambient CO_2 exceeds a threshold level, the differential between ambient and elevated CO_2 is constant:

$$\log(1+\beta) = E(B_{Eij}) - E(B_{Aij}) = \delta \quad (4)$$

These models can be interpreted as follows. The first model, in which α is expected to be positive and γ negative, implies that the magnitude of the CO_2 effect for LTLA (the difference between expected CO_2 effects at high and low CO_2) is related to the expected response at low CO_2:

$$\text{expected } CO_2 \text{ effect} = \alpha + \gamma\,(\text{expected response at ambient } CO_2)$$

Beta values are then calculated as follows:

$$\beta = \exp[\alpha + \gamma(\text{expected response at ambient } CO_2)] - 1$$

For the model characterizing the LTB responses, the interpretation of the CO_2 effect in the initial linear phase is the same as in the LTLA model. In the second phase, the CO_2 effect of size δ converts to a constant beta value of size $\beta = \exp(\delta) - 1$.

Model fitting

The non-linear parameter (γ) in these models was fitted by profile likelihood and the remaining parameters by least squares conditional on the maximum likelihood estimate of (γ). Both analyses were weighted to allow for the heteroscedasticity on the log scale as follows. The data (replication within each treatment combination) allowed modelling of the variance within each treatment combination using a generalized linear model with log link and Gamma distribution. Variances predicted from the best-fit model were used as weights for an analysis of the log-transformed leaf area and biomass data. The first issue in fitting the biomass model is deciding which responses should fall in the first and which in the second group. Strictly speaking, this is a change point problem where the form of the relationship changes from linear to constant at an unknown point that can be estimated. Exploratory analysis indicated the goodness-of-fit of the model was not very sensitive to the selection of the density × harvest combinations in the first and second phase, implying the change point would be very poorly estimated. The density × harvest combinations with the six lowest ambient level mean biomass (densities 1–5 for H1 and density 1 for H2) were selected for the first group and the remaining were put in the second group.

Results

EFFECTS OF CO_2, DENSITY AND HARVEST TIME ON GROWTH AND REPRODUCTION

Results of the weighted analysis of LTLA and LTB are presented in Table 1. To simplify the presentation of growth results, mean stand-level shoot biomass and total leaf area for each of the CO_2 × density × harvest combinations were predicted and back-transformed from the log scale, and are shown in Fig. 1. Estimated CO_2-induced growth enhancements (β-values) are also plotted above each pair of ambient and elevated CO_2 means.

Shoot biomass and leaf area of the stands were significantly increased by density ($P < 0.001$), but the effect of density on both biomass and leaf area decreased with time (D × H; $P < 0.001$; Table 1 and Fig. 1). At H1, for example, biomass increased approximately 16-fold across the density gradient, whereas by the final harvest there was at most a two-fold difference. Despite this convergence in stand biomass across the density gradient, it is important to note that for shoot biomass there was little indication that stands in either CO_2 concentration had reached a constant final yield. For leaf area, however, in the last harvest a ceiling of TLA did appear to have been reached at high density.

CO_2 significantly stimulated both shoot biomass and leaf area ($P < 0.001$), although β-values varied with harvest time and did so differently for the two measures of growth (Fig. 1 and Table 1). When averaged across all densities, β decreased with harvest time, decreasing from 0.88 to 0.32 for biomass and from 0.80 to −0.02 for leaf area (C × H; $P \leqslant 0.001$). At H1, β decreased with density for both shoot biomass and leaf area, but this pattern was more or less absent in H2 and H3. However, neither the CO_2–density interaction for leaf area nor the three-factor interaction for either variable was significant, suggesting that the underlying patterns apparent in Fig. 1 may be obscured by other non-significant contrasts in analyses involving terms with large degrees of freedom (5 for C × D and 10 for C × D × H).

At H3, density significantly increased the combined number of flowers and fruits produced per stand (Table 2; $P < 0.001$), an effect parallel to the growth response. The average magnitude of CO_2-induced enhancement of reproduction was quite similar to shoot biomass at H3 ($\beta = 0.38$ vs. 0.34, see below) and did not vary in a regular way with density treatments. For four of the six densities, reproductive β-values were statistically nonsignificant (Table 2).

Shoot RGR was considerably higher ($P < 0.05$) with elevated CO_2 at all densities during the growth

Table 1 *F*-values and associated *P*-values from weighted regression analysis of log(total biomass), LTB and log(total leaf area), LTLA

		Log (total biomass)		Log (total leaf area)	
Source	d.f.	*F*	*P*-value	*F*	*P*-value
Block	2	0.16	0.853	0.25	0.778
CO_2	1	50.03	<0.001	110.07	<0.001
Density	5	196.91	<0.001	121.03	<0.001
Harvest	2	943.19	<0.001	2510.09	<0.001
CO_2 × density	5	2.69	0.022	0.4	0.845
CO_2 × harvest	2	19.91	<0.001	6.81	0.001
Density × harvest	10	24.29	<0.001	25.27	<0.001
CO_2 × density × harvest	10	0.78	0.649	1.01	0.439
Residual	214 (213)	0.080		0.062	

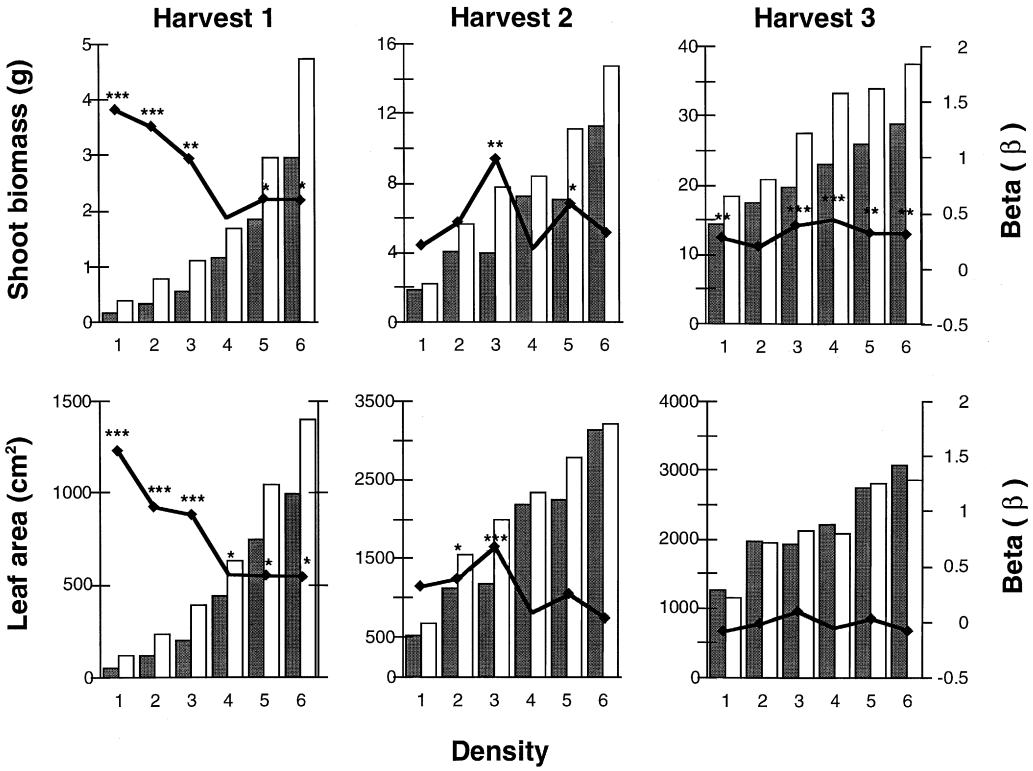

Fig. 1 Mean shoot biomass and standing leaf area of *Brassica kaber* grown at ambient (350 μl l^{-1}; light bars) and elevated (700 μl l^{-1}; shaded bars) CO_2 concentrations, at six densities (1, 2, 4, 8, 16 and 32 plants per pot), and measured at three stages of stand development. The magnitude of CO_2 enhancement (β) is also shown, with significance denoted as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

period H0–H1 (Table 3). For the period H1–H2, RGR was on average, higher in the ambient CO_2 treatments ($P < 0.05$), although these differences were small at higher densities. During the H2–H3 period, CO_2 effects were neither significant nor consistent across densities.

The response of root mass at H3 to CO_2 and density was very similar to that of shoot mass. Density significantly increased root mass ($P = 0.04$), and there was no evidence of a CO_2–density interaction ($P = 0.70$). Although the average β-value across all densities for root mass was 0.404, close to the β-value (i.e. 0.34) for shoots, the CO_2 effect for roots in the ANOVA model was not significant ($P = 0.139$). These results suggest that during the latter stages of *Brassica* stand development, shoot mass can be used to characterize the qualitative responses of whole plant mass to both CO_2 and density.

MODELLING RESULTS

The models relating β-values for shoot biomass or leaf area to standing shoot biomass and leaf areas at ambient CO_2 (350 μl l^{-1}) predicted the data very well

Table 2 Effects of CO_2 concentration and stand density on reproductive weight and total numbers of fruits and flowers per stand

	CO_2				
Density	350	700	β	t	Significance
Reproductive weight per stand					
1	0.585	0.970	0.66	1.85	$P < 0.1$
2	0.570	0.570	0.00	0.00	
4	0.877	1.149	0.31	0.92	
8	1.307	1.536	0.18	0.78	
16	1.117	1.589	0.42	1.60	
32	1.147	1.947	0.70	2.71	$P < 0.05$
SED CO_2*		0.295			
d.f.		15			
Reproductive number per stand density					
1	513	716	0.40	1.24	
2	523	651	0.24	0.55	
4	623	1070	0.72	1.93	$P < 0.1$
8	943	1099	0.17	0.67	
16	1166	1350	0.16	0.79	
32	919	1627	0.77	3.04	$P < 0.05$
SED CO_2*		232			
d.f.		7			

* SED for comparisons at density level 1 are those quoted divided by 1.4142.

Table 3 Relative growth rate (RGR) for shoot mass (g g^{-1} day^{-1}) in three interharvest periods for ambient and elevated CO_2 stands sown at six densities. See text for details on RGR estimation

Interharvest period	Harvest 0–1		Harvest 1–2		Harvest 2–3	
CO_2	L (350)	H (700)	L (350)	H (700)	L (350)	H (700)
RGR						
Density						
1	0.248	0.307	0.272	0.194	0.098	0.102
2	0.253	0.307	0.275	0.222	0.070	0.062
4	0.240	0.285	0.218	0.218	0.076	0.060
8	0.243	0.268	0.202	0.179	0.055	0.065
16	0.227	0.259	0.150	0.147	0.062	0.053
32	0.213	0.244	0.149	0.126	0.044	0.044
SED L vs. H	Dens 1	Dens > 1	Dens 1	Dens > 1	Dens 1	Dens > 1
	0.0133	0.0142	0.0314	0.0336	0.0057	0.0061

(Fig. 2a–d and Table 4) despite being based on few parameters. The addition of further terms and interactions (block × CO_2 + CO_2 × density × harvest) do not add to its explanatory power (Table 5).

Elevated CO_2 increased total leaf area by over 100% at small leaf areas but this enhancement gradually declined to zero at larger leaf areas (Fig. 2b,d). For small total shoot mass, elevated CO_2 had a similar effect to LTB, with β-values declining steeply with increasing biomass (Fig. 2a,c). However, beyond a threshold, β-values levelled off to about 34% ($\beta = \exp(\delta) - 1 = \exp(0.292) - 1 = 0.34$). The threshold ambient CO_2 biomass value at which this relationship levelled off was very low (< 2.5 g). Virtually all densities at both H2 and H3 were above this threshold.

Discussion

Few plants develop in the absence of neighbours. Consequently, it is essential that our understanding of plant responses to elevated CO_2, and the influences of these responses on community and ecosystem structure and function, account for the effects of density-dependent interactions. We began this study with a series of predictions regarding the density-dependent nature of plant responses to CO_2. First, we predicted that early in stand development, increasing density would have a negative effect on the magnitude of growth responses to CO_2. Secondly, we predicted that later in development, as standing biomass across all density treatments converged towards a higher

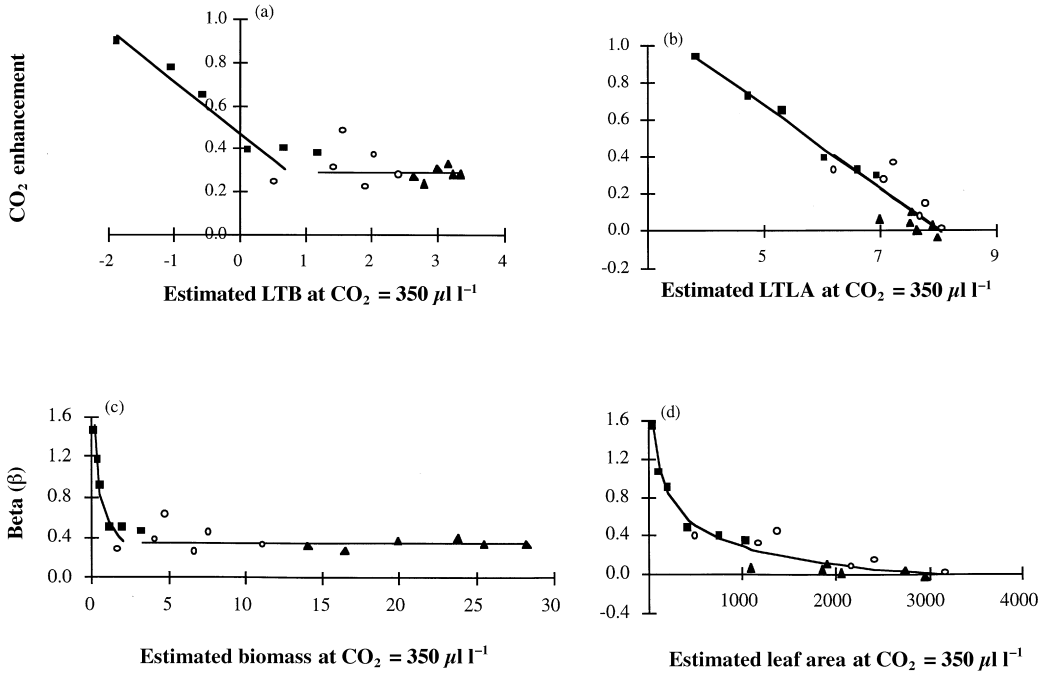

Fig. 2 Magnitude of CO_2-induced growth enhancement for stand mass and leaf area, estimated across a range of the plant stand densities and ages. (a) and (b), which present results on a logarithmic scale, depict CO_2 enhancement as the difference between elevated and ambient CO_2 values. (c) and (d), which present results on a non-transformed scale, depict CO_2 enhancement as the ratio of elevated to ambient CO_2 values (equivalent to β-values). Solid lines in each panel reflect values predicted from models (see text for details). Symbols in each plot are mean values for the 18 density by harvest combinations (H1 = squares; H2 = circles; H3 = triangles).

Table 4 Parameter estimates for the analysis of log (total stand shoot mass), LTB, and log (total stand leaf area), LTLA. Also presented are 95% confidence intervals

Parameter	LTB	SE or CI	LTLA	CI
α	0.474	0.329 to 0.619	1.78	1.72 to 1.84
γ	0.24	−0.394 to −0.071	−0.222	−0.274 to −0.162
δ	0.292	0.227 to 0.357		

Table 5 Statistics on goodness-of-fit of the models fitted to log (total stand shoot biomass), LTB, and log (total stand leaf area), LTLA, assuming the profile likelihood estimate of the slope γ. Shown are the residual mean squares for the models and the residual mean square for a fuller model that includes the model plus any further additional contribution of blocks, CO_2 and their interaction, and CO_2, density and harvest and their interactions. The test of the contribution of these additional terms is also included

Source	d.f.	SS	MS	*F*-ratio	*P*
Log (total stand shoot weight)					
Residual from model	230	23.34	0.1015		
Residual from model + block × CO_2 + CO_2 × density × harvest	210	21.06	0.1003		
Reduction due to block × CO_2 and CO_2 × density × harvest	20	2.28	0.114	1.14	NS
Log (total stand leaf area)					
Residual from model	230	18.21	0.07917		
Residual from model + block × CO_2 + CO_2 × density × harvest	211	16.70	0.07916		
Reduction due to block × CO_2 and CO_2 × density × harvest	19	1.51	0.07947	1.004	NS

yield and resource depletion limited plant growth and CO_2 responsiveness, the effects of density on CO_2 responsiveness would diminish. Both these predictions were supported. At H1, but not at H2 or H3, β-values for both shoot biomass and leaf area exhibited a regular and marked decline with increasing density.

Recent studies support our finding that β-values

decrease with density during early stages of development. Retuerto *et al.* (1996) found that after 40 days of growth, CO_2 stimulated growth by 86% for *Sinapis* plants in low density stands, but only by 15% at higher densities. A similar pattern (low density, $\beta = 0.49$, high density, $\beta = 0.14$) was reported by Wayne & Bazzaz (1995) for young birch seedlings. In a meta-analysis of CO_2 enrichment studies, Ackerly & Bazzaz (1995) found that in studies in which plants were raised without competition, 60 out of 63 species' trials exhibited positive growth responses to CO_2; however, in studies where species were subjected to competition, only 19 of 34 cases exhibited positive growth responses.

Most studies that have tested interactions between density and CO_2 have been relatively short-term, and based on one single harvest; thus we are not sure how transient or stable the reported β-values are. However, in one study with wheat (*Triticum aestivum* L., var. Capitole), in which changes of β with time were measured for plants growing at two densities, similar trends to those we observed with *Brassica* were reported (du Cloux *et al.* 1987). During the first 15 days of stand development, β-values in both low and high density averaged 0.45. Over the next 20 days, β-values for high density stands did not change; however, β-values for low density stands first rose to 0.70 and then declined back to values similar to high density stands (0.50). Results from a comparative study with eight annual species suggest that the effects of stand density and age on β-values may be species-specific (Thomas *et al.* 1999), with the density-dependence of β decreasing with time for some species but not others. Thus, while the responses of *Brassica* to the combined effects of CO_2, density and time were very marked, the limited number of comparable studies makes it difficult to assess how widespread and generalizable these results are.

REPRODUCTIVE VS. VEGETATIVE RESPONSES

The responses of final harvest yield (combined flower and fruit number) to the separate and combined effects of CO_2 and density did not differ markedly from those of biomass at final harvest. When averaged across all densities, β-values were only slightly greater for reproductive vs. vegetative growth (0.41 vs. 0.36). Quantifying reproductive responses on a mass basis did not change these results (Table 2; average $\beta = 0.38$). While significant differences between vegetative and reproductive responses to CO_2 are commonly reported for annual species (Garbutt & Bazzaz 1984; Ackerly & Bazzaz 1995; Farnsworth & Bazzaz 1995; Thomas *et al.* 1999), this is apparently not the case for *B. kaber*. From an agricultural perspective, these results suggest that, at least for some species, vegetative responses to CO_2 and density may be a good predictor of crop reproductive yield. However, from an evolutionary perspective, stand-level reproductive measures tell us very little about the potential effects of CO_2 and density on interindividual reproductive success or fitness. Recent research with annuals suggests that individual genotypes within competing populations can differ markedly in their reproductive responses to CO_2 and density (Bazzaz *et al.* 1995b). Such density-dependent effects of CO_2 enrichment on relative reproductive success and fitness can significantly influence the dynamics and microevolution of natural populations in future environments (Bazzaz *et al.* 1992; Geber & Dawson 1993; Curtis *et al.* 1994; Thomas & Jasienski 1996). These issues will be considered in detail for *B. kaber* in a later paper.

INSIGHTS FROM MODELLING

The interactive effects of stand density, CO_2 concentration and harvest time reduced to a simple pattern between β-values for both stand biomass and leaf area and predicted biomass or leaf area (respectively) in the ambient CO_2 treatments (our third prediction). This simple relationship was expected because during the earlier stages of stand development both increasing density and stand age result in greater biomass (Harper 1977). Nevertheless, it was surprising how little variation was observed in the relationship between β-values and stand developmental stage (i.e. size), and in the case of shoot biomass how consistent the threshold β-values were across so wide a range of stand mass. The biomass model indicates that, regardless of stand density or harvest time, β-values decline sharply with increasing stand shoot biomass up to a relatively low threshold ($< 10\ g\ m^{-2}$), and then level off at a β-value of 0.36. This β-value is very close to the average β-values reported in a number of broad surveys of agricultural and non-agricultural species (Kimball 1983; Cure & Acock 1986; Hunt *et al.* 1991; Lawlor & Mitchell 1991; Poorter 1993; Ceulmans & Mousseau 1994; Wullschleger *et al.* 1995). The leaf area model, in contrast, suggest that β-values decline more regularly and do not level off at a positive β-value, but approach zero in stands with the greatest foliage. The lack of a positive β-value for leaf area in the later stages of stand development reflects a decrease in leaf area ratio (data not shown), a response to elevated CO_2 commonly reported in the literature (Norby *et al.* 1992; Bazzaz 1993; Poorter *et al.* 1996; Roumet *et al.* 1996).

Although β-values for biomass decreased with increasing stand biomass, as predicted, it is important to note that the observed regular decrease and subsequent threshold values of β were not due to stands approaching a constant final yield (CFY). Even at the final harvest (H3), there was little indication that stand biomass was approaching an asymptotic ceiling. The consistent decline in RGR with increasing density in both CO_2 treatments does suggest interference, and RGR values did converge between the two CO_2 treat-

ments. However, across all treatments and harvests, RGR remained well above zero, indicating that stands had not reached CFY.

In summary, our study with *B. kaber* supports our predictions that density influences the magnitude and time–course of CO_2 responsiveness. Thus, estimates for species' or ecosystems' β-values must take into account this complexity, and limited reliance should be placed on β-values derived from individually grown plants. Our results also suggest that the density-dependence of β may be short-lived, with values converging across a broad range of stand developmental states. However, a number of important questions emerge from these observations that require further study. These include: (i) How long would the observed threshold β-values persist; does this constant phase of β-values change as stands do approach a constant final yield? (2) How widespread is this phenomenon among other species and with differing supplies of other resources (e.g. nitrogen and water)? (3) What are the physiological, developmental and population-level processes underlying such consistent responses? Future studies that link phenomenological descriptions of population-level responses to CO_2, with mechanistic measures, should be able to answer these important questions.

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